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The EGF/hnRNP Q1 axis is involved in tumorigenesis via the regulation of cell cycle-related genes

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Abstract

Heterogeneous nuclear ribonucleoprotein (hnRNP) Q1, an RNA-binding protein, has been implicated in many post-transcriptional processes, including RNA metabolism and mRNA splicing and translation. However, the role of hnRNP Q1 in tumorigenesis remains unclear. We previously performed RNA immunoprecipitation (RIP)-seq analysis to identify hnRNP Q1-interacting mRNAs and found that hnRNP Q1 targets a group of genes that are involved in mitotic regulation, including Aurora-A. Here, we demonstrate that altering the hnRNP Q1 level influences the expression of the Aurora-A protein, but not its mRNA. Stimulation with epidermal growth factor (EGF) enhances both binding between hnRNP Q1 and *Aurora-A* mRNA as well as the efficacy of the hnRNP Q1-induced translation of *Aurora-A* mRNA. The EGF/hnRNP Q1-induced translation of *Aurora-A* mRNA is mediated by the mTOR and ERK pathways. In addition, we show that hnRNP Q1 up-regulates the translation of a group of spindle assembly checkpoint (SAC) genes. hnRNP Q1 overexpression is positively correlated with the levels of Aurora-A and the SAC genes in human colorectal cancer tissues. In summary, our data suggest that hnRNP Q1 plays an important role in regulating the expression of a group of cell cycle-related genes. Therefore, it may contribute to tumorigenesis by up-regulating the translation of these genes in colorectal cancer.

Introduction

Genetic instability is a major event in the tumorigenesis of various cancers, including colorectal cancer. To maintain genome integrity and fidelity, it is important that cells regulate the expression of proteins involved in the cell cycle checkpoint mechanism, as well as control chromosome replication and separation during cell division[1]. Aurora-A is a serine/threonine kinase involved in regulating cell cycle progression, cell survival and malignant transformation[2]. Overexpression of Aurora-A has been found in many cancer cells, including breast, colon,

pancreas, liver, and stomach cancer[3]. The deregulation of Aurora-A kinase may result in chromosomal instability, indicating a link with tumorigenesis[4]. Aurora-A is regarded as an oncoprotein. The oncogenic role of Aurora-A has been frequently mentioned, and this protein has attracted substantial interest as a therapeutic target in various cancers[5]. Previous studies have suggested that the overexpression of Aurora-A in cancers may result from gene amplification[6,7], transcriptional up-regulation[8,9] or enhanced protein stability[10,11]. In our previous reports, we found that Aurora-A is translationally up-regulated by heterogeneous nuclear ribonucleoprotein Q1 (hnRNP Q1)[12], and epidermal growth factor (EGF) increases the expression of the Aurora-A protein via the ERK and Akt pathways[13]. However, the mechanism by which EGF mediates the translational up-regulation of Aurora-A expression and the translational regulatory factors that

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contribute to the increased expression of Aurora-A remains unclear.

hnRNPs are a family of RNA-binding proteins in eukaryotic cells. The hnRNP family comprises at least one or more RNA recognition motifs (RRMs) and combines with an RGG box or acidic domain for protein–protein interaction or RNA binding. hnRNPs are *trans*-acting elements that play an important role in multiple aspects of nucleic acid metabolism, including the packaging of nascent transcripts, alternative splicing and translational regulation[14]. Recently, two studies showed that aberrant hnRNP expression can cause a wide variety of diseases, including neurodegeneration and cancer[12,15]. Seven isoforms of hnRNP Q (also known as SYNCRIP or NSAP1) have been identified in human cells. These hnRNP Q isoforms (hnRNP Q1-Q7) result from the alternative splicing of a single gene, *SYNCRIP*. They prefer to bind to mRNAs with AU-rich RNA sequences. hnRNP Q contains three RRMs, one RGG-rich region (RGG box) and one acidic domain (AcD)[16,17]. Recent studies have shown that hnRNP Q plays a role in mRNA processing events, including pre-mRNA splicing, mRNA transport, and translational regulation[18–22]. hnRNP Q1 (*Homo sapiens*: NP_001153149.1) is the most abundant isoform of the hnRNP Q family. Unlike other members of the hnRNP Q family, which are expressed in the nucleus, hnRNP Q1 is primarily localized in the cytoplasm[19]. Cytoplasmic hnRNP Q1 may participate in mRNA localization, translation, and turnover. Consistent with this assumption, we previously demonstrated that hnRNP Q1 translationally up-regulates *Aurora-A* mRNA and increases tumorigenesis in colorectal cancer[12]. However, the role of hnRNP Q1 in tumorigenesis is not well characterized.

In the present study, we demonstrate that hnRNP Q1 acts as a *trans*-acting factor to up-regulate Aurora-A expression, and EGF enhances the hnRNP Q1-induced translational efficacy of *Aurora-A* mRNA. hnRNP Q1-induced *Aurora-A* mRNA translation might be mediated by the mTOR and ERK pathways. In addition to Aurora-A, hnRNP Q1 translationally up-regulates a group of genes known to be involved in the spindle assembly checkpoint (SAC). The expression of hnRNP Q1 is positively associated with the levels of Aurora-A and these SAC genes in colorectal cancer. In conclusion, we propose that hnRNP Q1 plays a novel role in tumorigenesis by translationally up-regulating cell cycle-related mRNAs in colorectal cancer.

Materials and methods
Cell culture, siRNA, and transfection

SW480 and HCT116 human colorectal cancer cells were grown in Leibovitz's L-15 medium (L-15; Invitrogen, Carlsbad, CA) and RPMI 1640 (Invitrogen), respectively. All media were supplemented with 10% fetal bovine serum (Invitrogen), 100 μg/ml streptomycin and 100 U/ml penicillin. The cells were maintained at 37 °C in 5% CO_2. *hnRNP Q* (*SYNCRIP*) siRNA was purchased from Dharmacon™ (L-016218) and was transfected into cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions.

Antibodies and reagents

Anti-EGFR (sc-03), anti-hnRNP Q (sc-56703), anti-phospho-ERK (sc-7383), anti-Lamin A/C (sc-7292), anti-ribosomal protein S6 (sc-74459), anti-Bub1 (sc-47743), Mps1 (sc-540), and anti-GAPDH (sc-25778) antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-ERK1/2 (06-182) antibody was purchased from Upstate (Charlottesville, VA, USA), and anti-Aurora-A (35C1) antibody was purchased from GeneTex (Irvine, CA, USA). Anti-BubR1 (#612502) antibody was purchased from Becton Dickinson (Franklin Lakes, New Jersey, USA), and anti-phospho-mTOR/Ser2448 (#2971), anti-p-4E-BP1/Thr70 (#9455) and anti-4E-BP1 (#9452) antibodies were purchased from Cell Signaling (Beverly, MA, USA). Anti-Aurora-B (A5102) antibody was purchased from Sigma-Aldrich (St. Louis, MO, USA). Anti-GFP (JL-8) antibody was purchased form Clontech (Mountain View, CA, USA). Human recombinant EGF was purchased from Prospec (Israel). U0126 was purchased from Sigma-Aldrich.

Quantitative real-time PCR (RT-qPCR)

Total RNA was isolated from cultured cells using Trizol reagent (Invitrogen) according to the manufacturer's instructions. Reverse transcription was performed using 1 μg of total RNA and a high-capacity cDNA Archive Kit (Applied BioSystems, Foster City, CA). Diluted cDNA (10 ng) was used as a template for RT-qPCR using the SYBR Advantage qPCR Premix (Bio-Rad, Hercules, CA). The relative expression of each mRNA was evaluated using the CFX Connect™ Real-Time PCR Detection System (Bio-Rad). *Actin* mRNA was used as the internal control. At least two independent analyses were performed for each sample. The following primer sequences were used: for *Aurora-A*, forward, 5′-AATGCCCTGTCTTACTGT-CATTC-3′ and reverse, 5′-TCCAGAGATCCACCTTCTC ATC-3′; for *Bub1*, forward, 5′-GGAGGACGCTCTGT-CAGCA-3′ and reverse, 5′-TCCAAAAACTCTTCAG-CATGAG-3′; for *BubR1*, forward, 5′-GAAGGACAAAAG ATCCTGGCTA-3′ and reverse, 5′-TGTGCTAAATCTGC TATACCAAACA-3′; for *Mad2*, forward, 5′-GGTCC TGGAAAGATGGCAG-3′ and reverse, 5′-ATCACTGAA CGGATTTCATCC-3′; for *Mps1*, forward, 5′-CCAGCG-CAGCTTTCTGTAG-3′ and reverse, 5′-TGTTCATTATG GAATCAATTGTCA-3′; and for *Actin*, forward, 5′-CTGGACT TCGAGCAAGAGATG-3′ and reverse, 5′-TGATGGAGTTGAAGGTAGTTTCG-3′.

Preparation of cell extracts and western blot analysis

The cells were harvested in RIPA lysis buffer (50 mM Tris-HCl/pH 8.0, 150 mM NaCl, 0.5% sodium deoxycholate, 1% Nonidet P-40, 0.1% SDS, 1 mM DTT, 10 mM β-glycerol phosphate, and 1 mM EGTA) containing a protease inhibitor cocktail. The protein lysates were cleared using centrifugation at 13,000 rpm for 10 min, and protein concentrations were determined using the Bradford method and a Protein Assay Kit (Bio-Rad). Equivalent protein lysates were used to perform western blot analysis using antibodies as indicated in the text. The levels of proteins were quantitated and normalized to the level of GAPDH using ImageJ software.

Ribosomal protein S6 immunoprecipitation assay (S6-IP)

The cells were harvested and lysed using S6-IP buffer (10 mM HEPES/pH 8.0, 100 mM KCl, 5 mM $MgCl_2$, 1% Triton-X100, 0.5% sodium deoxycholate, 100 units/ml RNase inhibitor, and 100 μg/ml cycloheximide). The cell lysates were pre-cleaned with protein A beads for 1 h at 4 °C and then immunoprecipitated using anti-ribosomal protein S6 antibodies for 2 h. Protein A beads were then added and incubated for additional 1 h at 4 °C. The beads were washed three times with S6-IP buffer, and the RNA immunoprecipitated by the beads was extracted with Trizol reagent (Invitrogen). The S6-bound mRNAs were evaluated using RT-qPCR.

RNA-immunoprecipitation assay (RIP)

The cell lysates for RIP were collected in RIP buffer (10 mM HEPES/pH 8.0, 40 mM KCl, 3 mM $MgCl_2$, 5% glycerol, 2 mM DTT, 0.5% sodium deoxycholate, 100 units/ml RNase inhibitor and protease inhibitor). The cytoplasmic extracts were incubated with anti-GFP antibody in RIP buffer at 4 °C for 2 h. The protein A/G beads were pre-cleaned with 1 mg/ml tRNA for 2 h and then added to the immunoprecipitated lysates. The mixture was then incubated at 4 °C for 2 h. The beads were washed three times with RIP buffer, and the RNA was isolated using Trizol reagent (Invitrogen) and processed for RT-qPCR.

In vivo translation assay

The in vivo translation assay was performed as previously described[13]. Firefly and renilla luciferase activities were detected using a Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA) according to the manufacturer's instructions.

Biotin pull-down assay

A biotinylated RNA probe was synthesized using an in vitro transcription system (Promega) according to the manufacturer's instructions. The cells were lysed using RIPA lysis buffer, and total cell lysates were pre-cleaned using streptavidin beads (Sigma-Aldrich) at 4 °C for 1 h. The lysates were then diluted with 5 × EMSA buffer (50 mM Hepes/pH 8.0, 250 mM KCl, 10 mM MgCl2, 5 mM DTT, 25% glycerol, 0.5 mg/ml tRNA, 0.7 mg/ml heparin, 100 unit/ml RNase inhibitor, and protease inhibitors) and incubated with biotin-labeled RNA probes at 4 °C for 2 h. The mixtures were subjected to UV-crosslinking (120 mJ/cm^2) three times for 5 min each and then incubated with streptavidin beads while rotating at 4 °C for 1 h. The beads were washed three times with wash solution (10 mM Hepes/pH 8.0, 40 mM KCl, 3 mM $MgCl_2$, 2 mM DTT, 5%, glycerol, 0.5% SDS and 2% NP-40) and then subjected to western blot analysis.

Immunohistochemistry (IHC)

Immunohistochemical staining was performed as previously described[13] using formalin-fixed and paraffin-embedded tissue sections. The sections were incubated with anti-EGFR (sc-03, Santa Cruz), anti-hnRNP Q1 (AP7852c, Abgent) or anti-Aurora-A (35C1, GeneTex) antibodies. The sections were then counterstained with hematoxylin.

Patient specimens

Clinical colorectal cancer specimens were used according to the tenets of the Declaration of Helsinki, and the laboratory protocol used in this study was approved by the Institutional Review Board (IRB) of National Cheng Kung University Hospital, Tainan, Taiwan (A-ER-100-403).

ONCOMINE microarray datasets

Microarray datasets for colorectal cancer (Kaiser Colon Dataset, 2007, GSE5206; Skrzypczak Colorectal 2 Dataset, 2010, GSE20916; Sabates-Bellver Colon Dataset, 2007, GSE8671; and Ki Colon Dataset, 2007, GSE6988) were accessed from the ONCOMINE Cancer Profiling Database (www.oncomine.org) and used to investigate the expression of *hnRNP Q1* and *Aurora-A* mRNA.

The Cancer Genome Atlas (TCGA) dataset

We downloaded TCGA colon adenocarcinoma and rectum adenocarcinoma datasets (version: 2015-02-24) via the UCSC Xena (http://xena.ucsc.edu). The gene expression profile was measured experimentally using the Illumina HiSeq 2000 RNA Sequencing platform of the University of North Carolina TCGA genome characterization center. These datasets show the gene-level transcription estimates, expressed as log_2-transformed RSEM normalized counts. The median age of the patients was 68 years (range, 31 to 90 years). Other clinicopathological information is shown in Supplementary Table 1. The mean level of *Aurora-A* expression was 10.02. The mean level of *hnRNP Q1* expression was 12.24. Values above the mean were considered high levels of *Aurora-A* and
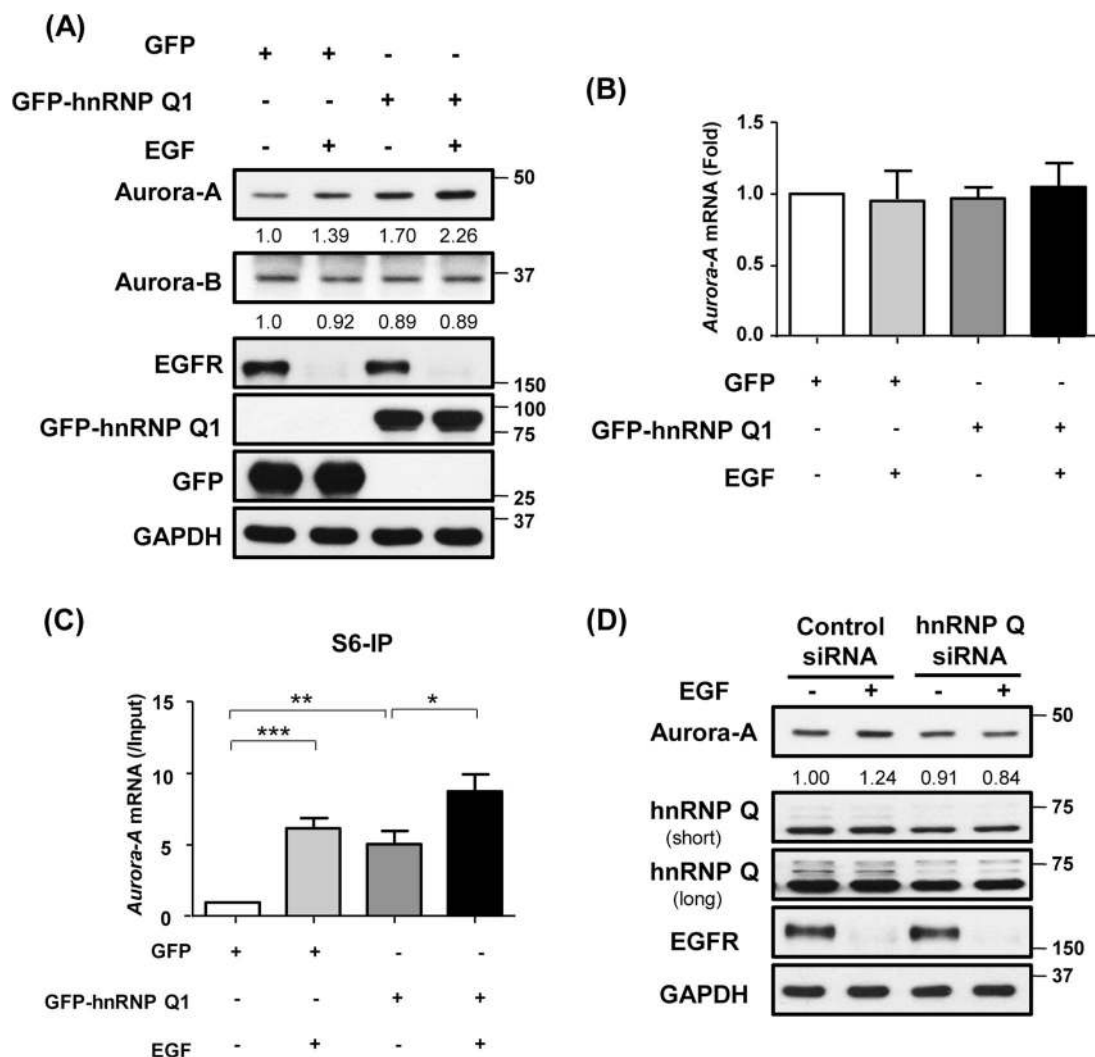
Fig. 1 EGF enhances the hnRNP Q1- mediated increase in *Aurora-A* mRNA translation. a SW480 cells were transfected with GFP or GFP-hnRNP Q1 and were incubated for 24 h. After serum starvation, cells were treated with EGF (10 nM) for 2 h, and total cell lysates were then harvested for western blot analysis. After EGF treatment, EGFR underwent endocytosis and ubiquitination[31–33]. **b** The levels of *Aurora-A* mRNA in GFP- and GFP-hnRNP-Q1-expressing SW480 cells were quantified following treatment with EGF. **c** Cell lysates were harvested for the ribosomal S6-IP assay. The amount of *Aurora-A* mRNA in the S6-IP complex was analyzed using RT-qPCR. The mean ± S.D. was obtained from three independent experiments (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). **d** The expression level of the Aurora-A protein in *hnRNP Q* siRNA-transfected SW480 cells treated with EGF was determined by western blot analysis. GAPDH was used as the loading control. The expression level of Aurora-A is shown as a ratio

hnRNP Q1 and those below the mean were considered low levels of *Aurora-A* and *hnRNP Q1*.

Statistical analysis

All statistical analyses were performed using GraphPad Prism (La Jolla, CA, USA) and SPSS 17.0 software (SPSS, Chicago, IL, USA). All experimental data were expressed as the means ± S.D. Two-tailed *t*-test was used to compare means between two groups. One-way ANOVA was used to compare means among three groups. The Chi-square test and Spearman's correlation coefficient were used to analyze the clinical specimen data. Significance was accepted at $p < 0.05$.

Results

EGF and hnRNP Q1 cooperatively enhance the translation of *Aurora-A* mRNA

Our previous report indicated that, in colorectal cancer, EGF increases Aurora-A protein expression by enhancing the translational efficiency of *Aurora-A* mRNA via ERK and Akt pathways[13]. However, the regulatory factors involved in the EGF-induced translational

up-regulation of *Aurora-A* mRNA remain unknown. Recently, we found that hnRNP Q1, an RNA-binding protein, can bind to and translationally up-regulate *Aurora-A* mRNA[12]. Therefore, we sought to further examine whether the EGF signaling pathway also contributes to the hnRNP Q1-mediated translational up-regulation of *Aurora-A* mRNA. To address this

question, we transiently transfected cells with GFP or GFP-hnRNP Q1, and the total cell lysates were then collected to determine the expression level of Aurora-A. The results showed that hnRNP Q1 and EGF each increases the protein level of Aurora-A and that treating cells with both EGF and hnRNP Q1 result in a synergistic effect on Aurora-A expression. The
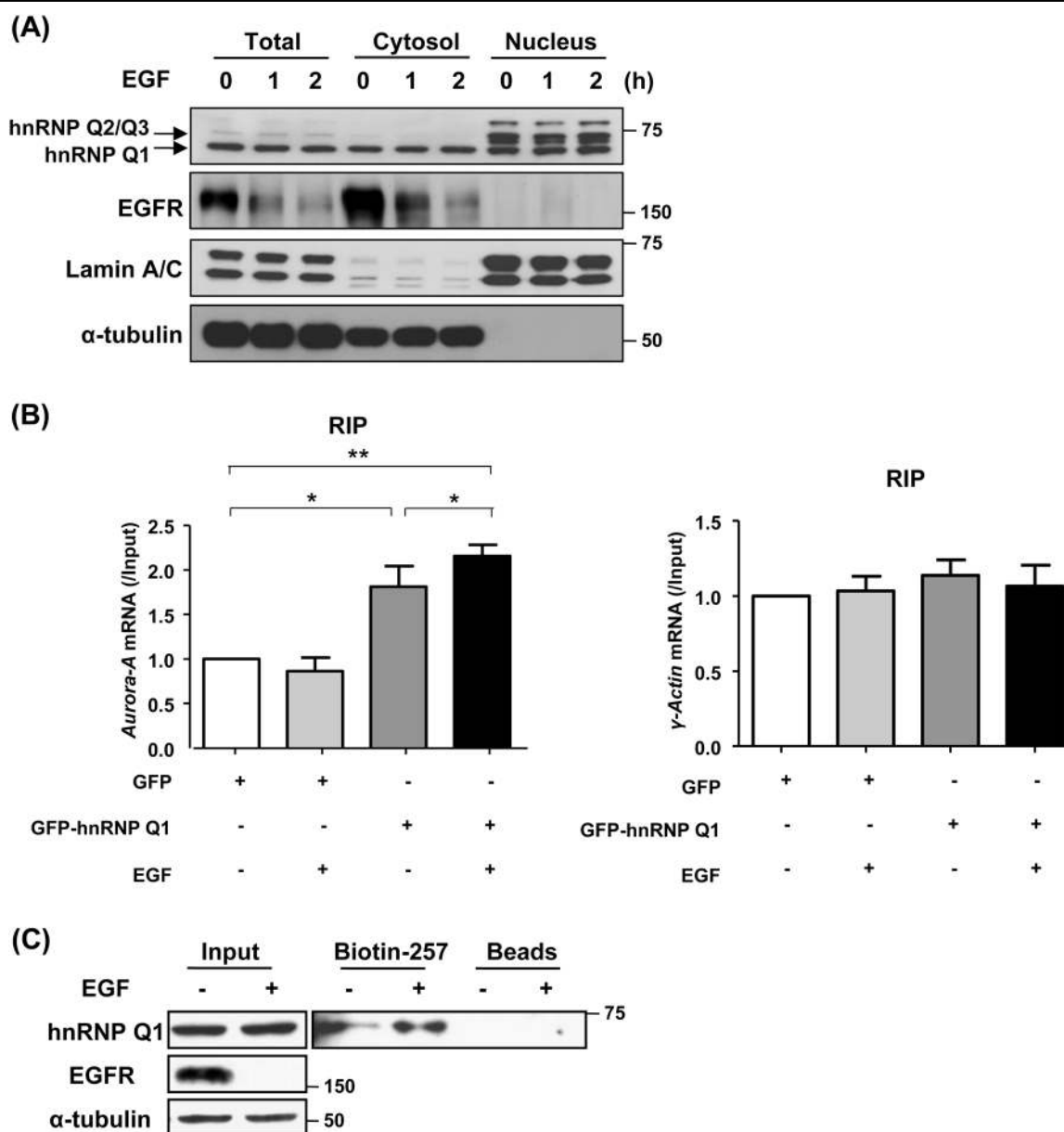

Fig. 2 EGF enhances the ability of hnRNP Q1 to bind to the *Aurora-A* mRNA 5′-UTR. a Total cell lysate, the cytosolic and nuclear fractions were obtained from SW480 cells after treatment with 10 nM EGF and were used to perform western blot analysis with the indicated antibodies. **b** Cytoplasmic extracts obtained from GFP- or GFP-hnRNP Q1-expressing cells treated with EGF were used to perform RIP using anti-GFP antibodies. The bound *Aurora-A* mRNA was measured using RT-qPCR. *γ-Actin* was used as the negative control for RIP. The mean ± S.D. was obtained from three independent experiments ($*p < 0.05$; $**p < 0.01$). **c** SW480 cells were treated with (+) or without (−) EGF for 2 h, and then total cell lysates were harvested to perform biotin-pull down assays using the biotin-labeled *Aurora-A* mRNA 5′-UTR 257 nt probe (Biotin-257). Beads were used as the negative control
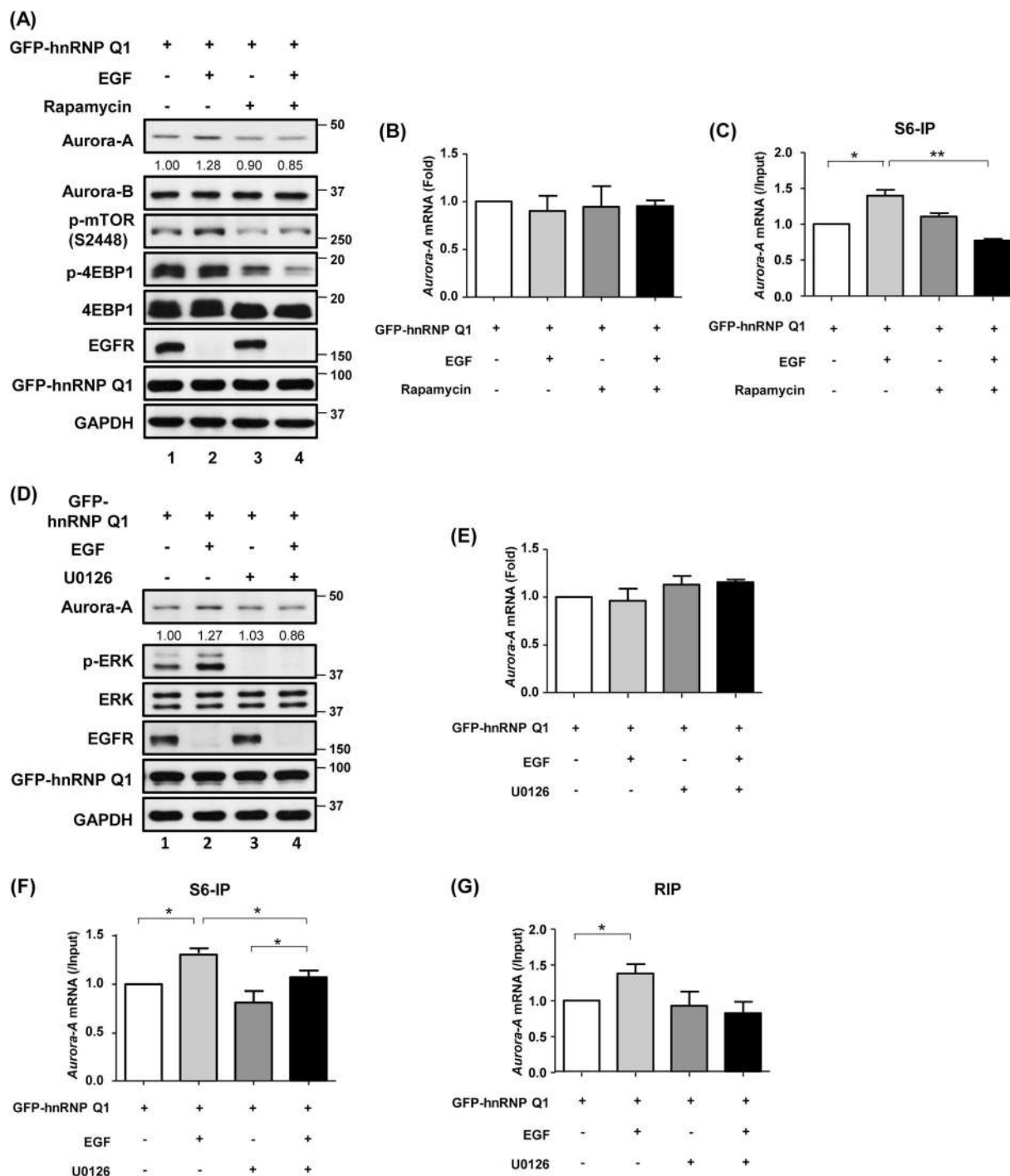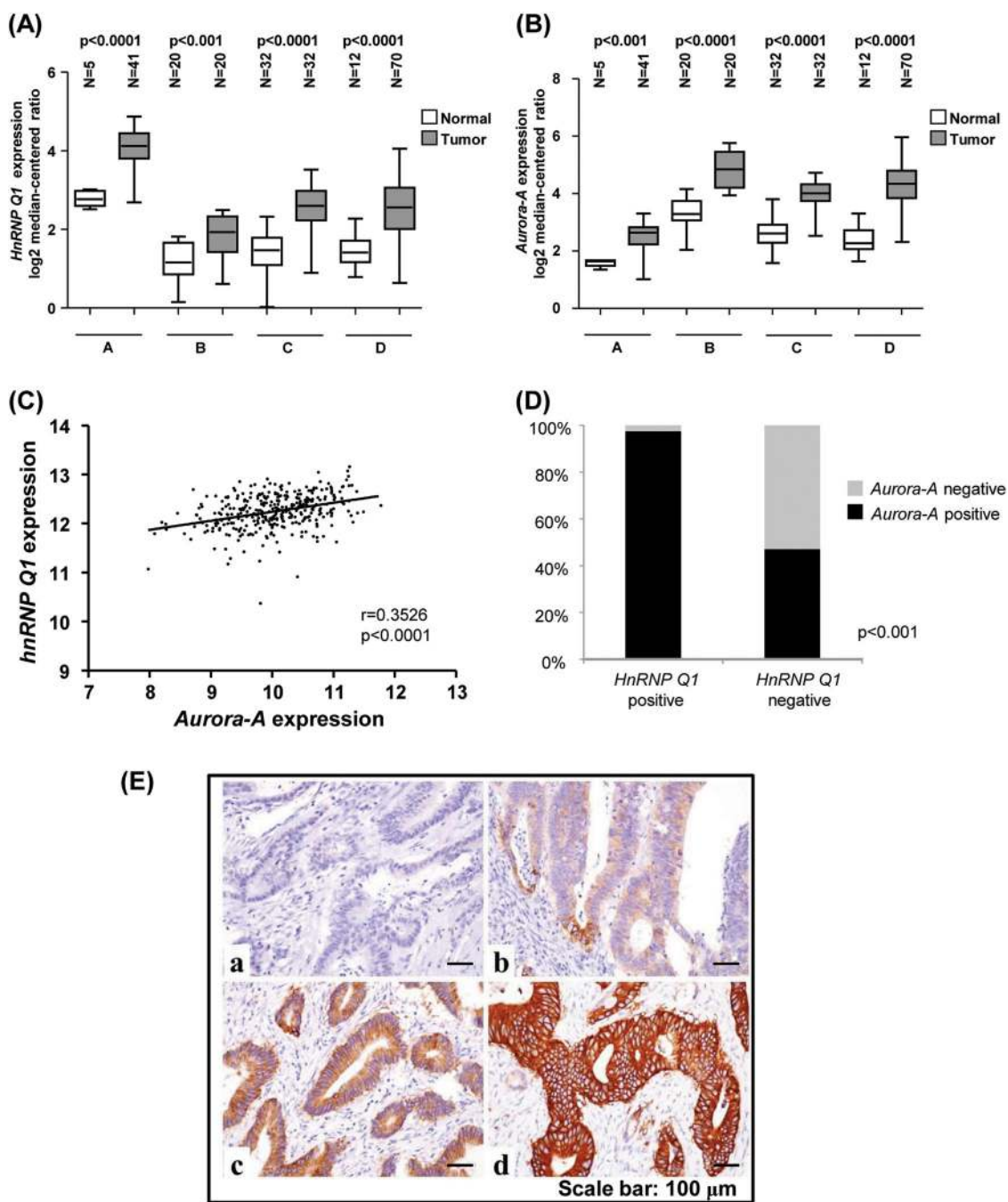
Fig. 3 The mTOR and ERK pathways regulate the hnRNP Q1-induced translation of *Aurora-A* mRNA following stimulation with EGF.
a SW480 cells were transfected with GFP-hnRNP Q1 for 24 h. Serum-starved cells were pre-treated with rapamycin (100 nM) for 2 h and then were treated with EGF (10 nM) for an additional 2 h. The level of Aurora-A protein was evaluated using western blot analysis and was quantified as a ratio. **b** Quantification of the expression level of *Aurora-A* mRNA in GFP-hnRNP Q1-expressing cells treated with rapamycin and EGF, as described in (**a**). **c** The translational efficiency of the *Aurora-A* mRNA was determined using S6-IP and was quantitated using RT-qPCR. **d, e** GFP-hnRNP Q1-expressing SW480 cells were serum-starved and then pre-treated with U0126 (10 μM) for 30 min followed by treatment with EGF for another 2 h. Cell lysates were collected to determine the protein expression of Aurora-A by western blot analysis (**d**) and the mRNA expression of *Aurora-A* using RT-qPCR (**e**). **f, g** GFP-hnRNP Q1-expressing cells were treated with U0126 and then EGF, as described above. The cell lysates were then collected for S6-IP (**f**) or RIP assays using anti-GFP antibodies (**g**). The bound *Aurora-A* mRNA was measured using RT-qPCR. The mean ± S.D. was obtained from three independent experiments. (*$p < 0.05$; **$p < 0.01$)

Fig. 4 (See legend on next page.)

Fig. 4 hnRNP Q1 expression is positively correlated with Aurora-A expression in human colorectal cancer. a, **b** Four cohorts of colorectal cancer from the "Oncomine" public database (https://www.oncomine.org) were used to analyze the expression patterns of *hnRNP Q1* (*SYNCRIP*, **a**) and *Aurora-A* (**b**). **c** Spearman's correlation was used to analyze the relationship between *Aurora-A* and *hnRNP Q1* expression in clinical colorectal cancer samples obtained from the TCGA dataset. **d** The expression levels of the *hnRNP Q1* and *Aurora-A* mRNAs were quantitated using RT-qPCR in 56 paired colorectal cancer and normal tissue samples, and the correlation between their expression levels was analyzed using Pearson's Chi-square analysis ($p < 0.001$). **e** Colorectal cancer tissues were collected to analyze the expression of EGFR by IHC in 28 tissues that overexpressed Aurora-A and hnRNP Q1 and 26 tissues that expressed low levels of Aurora-A and hnRNP Q1. Representative images demonstrate the intensity of EGFR as follows: **a** score = 0, no staining visible; **b** score = 1, weak membranous staining in <10% of all tumor cells; **c** score = 2, moderate continuous membranous staining in >10% of all tumor cells; and **d** score = 3, strong and continuous membranous staining in >10% of all tumor cells. Positive staining ≥30% indicated high levels of Aurora-A and hnRNP Q1, and scores <30% indicated low levels of Aurora-A and hnRNP Q1. The results of the IHC were used to determine the correlation among EGFR, Aurora-A and hnRNP Q1 expression by Pearson's Chi-square analysis, $p = 0.046$

level of the homologous protein Aurora-B was not affected in hnRNP Q1-expressing or EGF-treated cells (Fig. 1a and Supplementary Figure S1A). These results indicate that hnRNP Q1 may be involved in the EGF-mediated translational up-regulation of *Aurora-A* mRNA, and this effect is specific to *Aurora-A*. Quantitative real-time PCR (RT-qPCR) was performed to demonstrate that neither hnRNP Q1 nor EGF treatment affected *Aurora-A* mRNA expression (Fig. 1b and Supplementary Figure S1B).

To further evaluate the effect of hnRNP Q1 and EGF on the up-regulation of the translational efficiency of *Aurora-A* mRNA, we performed a ribosomal protein S6-immunoprecipitation assay (S6-IP) using hnRNP Q1-expressing cells. The results showed that the S6 subunit-associated *Aurora-A* mRNA was increased in hnRNP Q1-overexpressing or EGF-treated cells (Fig. 1c), and hnRNP Q1 enhanced the translational efficacy of *Aurora-A* mRNA in response to EGF treatment (Fig. 1c and Supplementary Figure S1C). To determine the contribution of hnRNP Q1 to the EGF-mediated translational up-regulation of *Aurora-A* mRNA, an hnRNP Q siRNA was used to knock down endogenous hnRNP Q1 expression. We then examined the expression of Aurora-A using western blot analysis. The data showed that knocking down hnRNP Q1 abolished the EGF-enhanced protein expression of Aurora-A (Fig. 1d and Supplementary Figure S1D). This result implies that the EGF-enhanced expression of Aurora-A is hnRNP Q1 dependent. These results demonstrate that hnRNP Q1 regulates the translation of *Aurora-A*, and the EGF signaling pathway may play an important role in controlling the hnRNP Q1-mediated translational regulatory effect on Aurora-A expression in colorectal cancer cells.

EGF enhances the ability of hnRNP Q1 to bind to the *Aurora-A* mRNA 5′-UTR

According to a previous report, hnRNP Q1 is distributed throughout the cell but is localized primarily in the cytoplasm[19]. Here, we investigated whether EGF alters the subcellular localization of hnRNP Q1. The results

revealed that EGF did not affect the expression or subcellular localization of hnRNP Q1 (Fig. 2a). Our previous report indicated that hnRNP Q1 directly binds to the *Aurora-A* mRNA 5′-UTR at its RBD2 and RBD3 domains[12]. Therefore, we used an RIP to evaluate the capacity of GFP-hnRNP Q1 to bind to the *Aurora-A* mRNA following stimulation with EGF. As expected, GFP-hnRNP Q1 was associated with *Aurora-A* mRNA, and EGF treatment enhanced its ability (Fig. 2b). γ-*Actin*, a gene that is not targeted by hnRNP Q1[22], was used as the negative control for RIP. A biotin pull-down assay was then performed using the biotin-labeled *Aurora-A* mRNA 5′-UTR 257 nt RNA probe to demonstrate the direct interaction between hnRNP Q1 and *Aurora-A* mRNA[13]. The results showed that endogenous hnRNP Q1 interacted with the *Aurora-A* mRNA 5′-UTR 257 nt RNA probe, and EGF enhanced this interaction (Fig. 2c). These results indicate that hnRNP Q1 acts as a *trans*-acting factor to translationally up-regulate the *Aurora-A* mRNA, and EGF enhances the binding ability and translational efficacy of hnRNP Q1.

EGF increases the hnRNP Q1-mediated translational up-regulation of *Aurora-A* mRNA through the mTOR and ERK pathways

Our previous data indicated that EGF translationally regulates *Aurora-A* mRNA through the ERK and Akt pathways in colorectal cancer[13]. Here, we sought to determine whether the ERK and Akt pathways contribute to the hnRNP Q1-mediated translational up-regulation of *Aurora-A* mRNA. We first analyzed the cap-dependent/mTOR signaling pathway. Similar to the results of a previous report[13], we found that EGF enhanced Aurora-A expression and mTOR activation in GFP-hnRNP Q1-ovexpressing cells (Fig. 3a, compare lanes 1 and 2). The addition of rapamycin, a mTOR inhibitor, blocked the EGF-mediated GFP-hnRNP Q1-enhanced expression of Aurora-A (Fig. 3a, compare lanes 1, 2, and 4). Neither EGF nor rapamycin treatment altered *Aurora-A* mRNA levels in GFP-hnRNP Q1-expressing cells (Fig. 3b). This result suggested that the mTOR pathway might

Table 1   The correlation between clinicopathological variables and the expression of hnRNP Q1 in data obtained from the TGCA dataset

Clinical variables	HnRNP Q high	HnRNP Q low	*p* Value[a]
Age			0.996
<65	80 (52.6%)	72 (47.4%)	
≥65	101 (52.6%)	91 (47.4%)	
Gender			0.313
Male	102 (55.1%)	83 (44.9%)	
Female	79 (49.7%)	80 (50.3%)	
Aurora-A			<0.001
High	118 (65.6%)	63 (38.4%)	
Low	62 (34.4%)	101 (61.6%)	
MMR			0.832
dMMR	27 (54.0%)	23 (46.0%)	
pMMR	154 (52.4%)	140 (47.6%)	
TNM stage			
T0	0 (0.0%)	1 (100.0%)	0.235
T1	3 (33.3%)	6 (66.7%)	
T2	28 (50.9%)	27 (49.1%)	
T3	132 (55.9%)	104 (44.1%)	
T4	18 (52.6%)	25 (58.1%)	
N0	103 (53.4%)	90 (46.6%)	0.310
N1	51 (56.7%)	39 (43.3%)	
N2	27 (44.3%)	34 (55.7%)	
M0	158 (52.8%)	141 (47.2%)	0.828
M1	23 (51.1%)	22 (48.9%)	
Stage			0.941
0–2	97 (52.4%)	88 (47.6%)	
3–4	84 (52.8%)	75 (47.2%)	
Site			0.014
Colon	127 (48.8%)	133 (51.2%)	
Rectum	54 (64.3%)	30 (35.7%)	

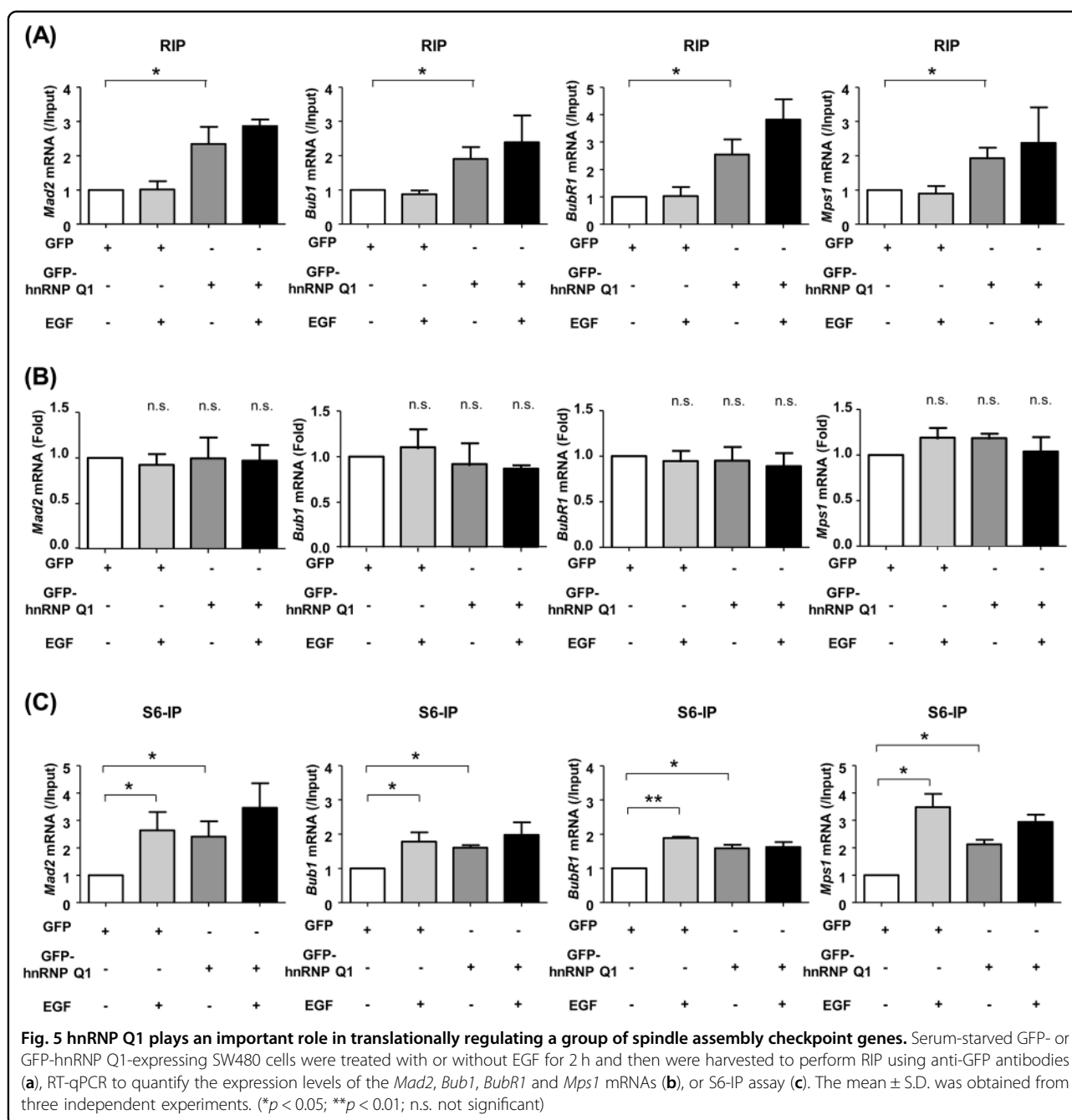[a]The data were analyzed using Pearson's chi-square test

Table 2   The correlation between clinicopathological variables and the expression of Aurora-A in data obtained from the TGCA dataset

Clinical variables	Aurora-A high	Aurora-A low	*p* Value[a]
Age			0.919
<65	80 (52.6%)	72 (47.4%)	
≥65	100 (52.1%)	92 (47.9%)	
Gender			0.634
Male	99 (53.5%)	86 (46.5%)	
Female	81 (50.9%)	78 (79.1%)	
HnRNP Q			<0.001
High	118 (65.6%)	62 (34.4%)	
Low	63 (38.4%)	101 (61.6%)	
MMR			<0.001
dMMR	15 (30.0%)	35 (70.0%)	
pMMR	165 (56.1%)	129 (43.9%)	
TNM stage			
T0	0 (0.0%)	1 (100.0%)	0.018
T1	6 (66.7%)	3 (33.3%)	
T2	33 (60.0%)	22 (40.0%)	
T3	128 (54.2%)	108 (45.8%)	
T4	13 (30.2%)	30 (69.8%)	
N0	112 (58.0%)	81 (42.0%)	0.019
N1	45 (50.0%)	45 (50.0%)	
N2	23 (37.7%)	38 (62.3%)	
M0	162 (54.2%)	137 (45.8%)	0.076
M1	18 (40.0%)	27 (60.0%)	
Stage			0.046
0–2	106 (57.3%)	79 (42.7%)	
3–4	74 (46.5%)	85 (53.5%)	
Site			0.129
Colon	130 (50.0%)	130 (50.0%)	
Rectum	50 (59.5%)	34 (40.5%)	

[a]The data were analyzed using Pearson's chi-square test

contribute to the hnRNP Q1-mediated regulation of *Aurora-A* mRNA translation, and EGF enhanced this effect by modulating the mTOR pathway. In addition, the EGF-enhanced recruitment of ribosomal protein S6 to *Aurora-A* mRNA was abolished by rapamycin treatment in GFP-hnRNP Q1-expressing cells (Fig. 3c). These results indicate that hnRNP Q1 translationally increases Aurora-A expression via the mTOR pathway.

Next, the ERK pathway was examined to evaluate its involvement in the hnRNP Q1-mediated translation of *Aurora-A* mRNA. As shown in Fig. 3d, adding U0126, an ERK inhibitor, eliminated the effect of EGF on the hnRNP Q1-enhanced expression of the Aurora-A protein (Fig. 3d, compare lanes 1, 2, and 4) but did not affect the level of *Aurora-A* mRNA (Fig. 3e). The EGF-enhanced association of ribosomal protein S6 with *Aurora-A* mRNA (Fig. 3f)

Fig. 5 hnRNP Q1 plays an important role in translationally regulating a group of spindle assembly checkpoint genes. Serum-starved GFP- or GFP-hnRNP Q1-expressing SW480 cells were treated with or without EGF for 2 h and then were harvested to perform RIP using anti-GFP antibodies (**a**), RT-qPCR to quantify the expression levels of the *Mad2*, *Bub1*, *BubR1* and *Mps1* mRNAs (**b**), or S6-IP assay (**c**). The mean ± S.D. was obtained from three independent experiments. (*$p < 0.05$; **$p < 0.01$; n.s. not significant)

and the binding of hnRNP Q1 to *Aurora-A* mRNA (Fig. 3g) were also abolished in cells treated with U0126.

hnRNP Q1 expression is positively correlated with Aurora-A levels in human colorectal cancer

Previous reports have indicated that hnRNP Q1 may be aberrantly overexpressed in cancers[12,15], and our results showed that hnRNP Q1 translationally up-regulates Aurora-A protein expression. These findings collectively suggest that the level of hnRNP Q1 may be increased in cancer cells in parallel with the Aurora-A expression, which is regulated by hnRNP Q1. To address this issue, we analyzed the expression of *hnRNP Q1* mRNA in colorectal cancer. We first tested cancer microarray datasets that are publicly available from Oncomine (https://www.oncomine.org) to survey many colorectal cancer specimens. We found that the relative levels of both the *hnRNP Q1* and *Aurora-A* mRNAs were significantly higher in colorectal cancer tissues than in normal tissues across four different cohorts (Fig. 4a, b). We also analyzed the correlation between the *hnRNP Q1* and *Aurora-A* mRNA levels in colorectal cancer using The Cancer Genome
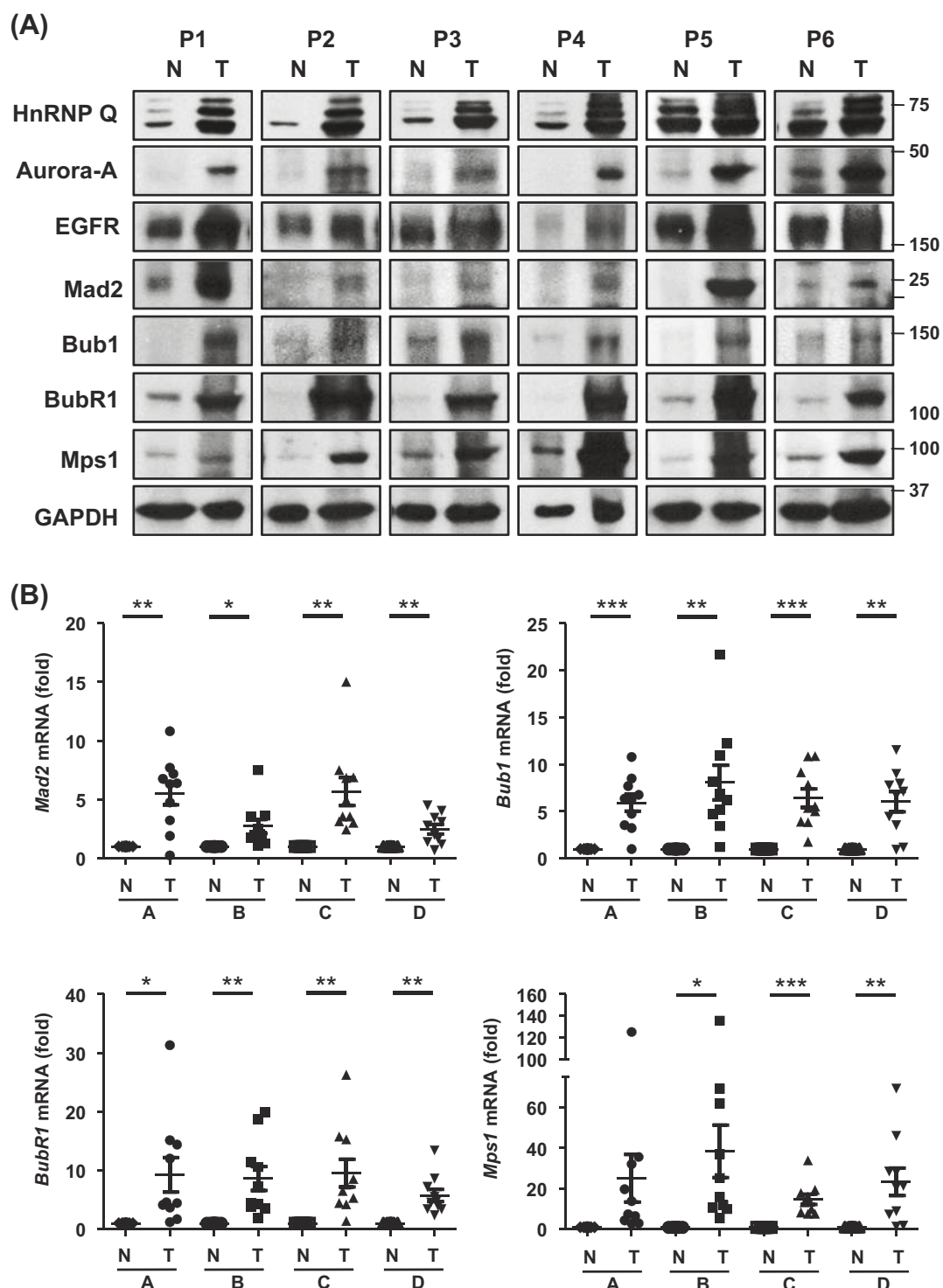
Fig. 6 Parallel changes in the overexpression of EGFR, hnRNP Q1, Aurora-A and spindle assembly checkpoint genes in colorectal cancer tissues. a Western blot analysis was used to determine the expression levels of the EGFR, hnRNP Q1, Aurora-A, Mad2, Bub1, BubR1, and Mps1 proteins in human colorectal tumors (T) and adjacent normal colon mucosal tissues (N). Six representative specimens (P1–P6) are shown. **b** The expression levels of the *Mad2*, *Bub1*, *BubR1*, and *Mps1* mRNAs in human colorectal cancer tissues (T) and their normal colon mucosal tissues (N) were analyzed using RT-qPCR. ($N = 40$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$)

Atlas (TCGA) dataset. As shown in Fig. 4c, there was a significant positive correlation between the levels of the *hnRNP Q1* and *Aurora-A* mRNA in colorectal cancer tissues (Spearman's correlation: $r = 0.3563$; $p < 0.0001$). A clinicopathological analysis indicated that *hnRNP Q1* is overexpressed in colorectal cancer and is significantly correlated with *Aurora-A* expression ($p < 0.001$). *hnRNP Q1* was detected more often in rectal carcinomas than in the carcinomas of the proximal colon (64.3 vs 48.8%, $p = 0.014$) (Table 1). *Aurora-A* is significantly correlated with MMR ($p < 0.001$) and TNM stage (T stage: $p = 0.018$; N stage: $p = 0.019$) (Table 2). The expression of *Aurora-A* is closely associated with the tumor stage, and it is more frequently found in the early stage of colorectal cancer (57.3 vs 46.5%; $p = 0.046$). *hnRNP Q1* and *Aurora-A* expression is not significantly correlated with age and gender.

To further evaluate the correlation between the EGFR signaling pathway and hnRNP Q1-regulated expression of Aurora-A, 54 paired colorectal cancer tissues were collected to analyze the expression patterns of Aurora-A, hnRNP Q1, and EGFR. Consistent with our previous findings, the results of RT-qPCR indicated that the expression levels of *hnRNP Q1* and *Aurora-A* are positively correlated (Pearson's Chi-square analysis, $p < 0.001$) (Fig. 4d). Our previous study indicated that hnRNP Q1 and Aurora-A are co-expressed in 69.6% (78/129) of colorectal cancer tissues (ref. [12] and Supplementary Figure S2). Here, we observed that the high expression level of both hnRNP Q1 and Aurora-A was positively correlated with the high level of EGFR (Pearson's Chi-square analysis, $p = 0.046$) (Fig. 4e). Overall, these results indicate that the expression of hnRNP Q1 is associated with Aurora-A protein expression in colorectal cancer; an increased level of *Aurora-A* mRNA may augment the effect of hnRNP Q1 on its translational regulation, and the EGFR pathway may be involved in the hnRNP Q1-mediated translational up-regulation of *Aurora-A* mRNA.

hnRNP Q1 plays an important role in regulating the expression of a group of SAC genes

We previously used RIP analysis followed by next-generation sequencing (NGS) to show that a group of SAC genes are potential targets of hnRNP Q1 (GEO accession number GSE76457[12]). However, the NGS results were preformed using GFP-hnRNP Q1-over-expressing cells that are not treated with EGF. Here, we evaluated the effect of hnRNP Q1 and EGF on the translational regulation of the expression of these SAC genes. Four genes, including *Mad2, Bub1, BubR1,* and *Mps1*, were chosen for further analysis. The results of RIP indicated that hnRNP Q1 binds to the *Mad2, Bub1, BubR1,* and *Mps1* mRNAs, and EGF only slightly enhances the binding ability of GFP-hnRNP Q1 to these

four genes (Fig. 5a). There were no obvious changes in the expression of these four genes at the mRNA level in either GFP-hnRNP Q1-overexpressing or EGF-treated cells (Fig. 5b). An S6-IP assay further demonstrated that while hnRNP Q1 translationally up-regulated the expression of these four genes, EGF slightly increased the hnRNP Q1-mediated translation of these genes (Fig. 5c). These data suggest that hnRNP Q1 is an important regulator in translationally up-regulating SAC genes and Aurora-A in colorectal cancer; EGF is involved in hnRNP Q1-modulated *Aurora-A* mRNA translation and may participate in hnRNP Q1-mediated *SAC* mRNA translation. A more detailed mechanism regarding the effect of EGF treatment in hnRNP Q1-mediated SAC mRNAs translational regulation needs to be further investigated.

Co-expression of SAC proteins, EGFR, and Aurora-A in human colorectal tissues

The expression of SAC genes in human colorectal cancer tissues was further evaluated using western blot analysis and RT-qPCR. The results showed that the expression levels of the Mad2, Bub1, BubR1, and Mps1 proteins and mRNAs were significantly higher in colorectal cancer tissues than in paired adjacent normal tissues (Fig. 6a, b). Next, we investigated the correlation between overexpressed Mad2, Bub1, BubR1, and Mps1 with hnRNP Q1 in human colorectal tissues. However, due to the difficulty in obtaining the protein expression profile from the public database, and because overexpressed mRNA can also contribute to increased protein expression levels through up-regulating translational efficiency, we analyzed the mRNA expression pattern of these genes using the TCGA dataset. Data from the TCGA dataset support the positive correlation between *hnRNP Q1* and *Mad2, Bub1, BubR1,* and *Mps1* mRNAs in colorectal cancer (Fig. 6b and Supplementary Figure S3). These results suggest that EGF signaling may modulate hnRNP Q1 to increase the expression of Aurora-A and SAC proteins to promote the development of colorectal cancer.

Discussion

The dysregulation of Aurora-A plays a vital role in tumorigenesis. It has been shown that the overexpression of Aurora-A can be achieved through gene amplification, RNA transcriptional activation, or increased protein stability. No previous reports have indicated that Aurora-A is naturally deficient in human tumors[23]. In our previous report, we found that EGF increases the expression of the Aurora-A protein via the translational regulatory machinery. These data suggested a potential explanation for the abnormal expression of Aurora-A in cancers[13]. Recently, we found that a novel *trans*-acting factor, hnRNP Q1, regulates the translation of *Aurora-A* mRNA by binding to its 5′UTR[12]. In this report, we used IHC,

western blot analysis, and RT-qPCR to show that hnRNP Q1 translationally up-regulates *Aurora-A* mRNA and the overexpression of Aurora-A is positively correlated with the overexpression of hnRNP Q1 in colorectal cancer tissues.

The results of RIP showed that EGF enhances the binding ability of hnRNP Q1 to *Aurora-A* mRNA. We speculate that EGF may play a role in post-translationally modifying hnRNP Q1 to influence its ability to bind to its target mRNAs, thereby enhancing its translational efficiency. Indeed, previous reports have suggested that EGF controls the functions of many hnRNPs through post-translational modifications. For example, EGF treatment led to the ubiquitylation of hnRNP A1 and was associated with cell migration;[24] EGF activates SR proteins, which are RNA-binding proteins, via Akt-SR protein kinase (SRPK) to regulate *Rac1b* mRNA splicing;[25] the phosphorylation of the ARE-binding protein DAZAP1 [DAZ (deleted in azoospermia)-associated protein 1] by EGF/ERK2 may contribute to the induction of translation;[26] and EGF induces the acetylation of hnRNP A1 and hnRNP L in colorectal cancer cells[27]. However, more evidence is needed to determine whether EGF controls the post-translational modification of hnRNP Q1.

The activation of EGFR may also play an important role in chromosome instability[28]. It was previously reported that EGF induces centrosome separation and promotes mitotic progression by activating Akt[29], and the EGFR/MEK/ERK/CDK5 pathway induces p-FAK/Ser732, which promotes mitosis and cell proliferation[30]. These findings indicate that EGF regulates mitotic progression by initiating signaling transduction but does not reveal its specific molecular mechanism. In this study, we provide the first evidence to demonstrate the correlation between the expression of EGFR and SAC proteins in colorectal cancer tissues (Fig. 6b) and that EGF signaling might be an important regulator of the augmented expression of SAC genes. In conclusion, these data indicate that hnRNP Q1 up-regulates the expression levels of Aurora-A (this study and[12]) and a group of SAC genes, and the expression of hnRNP Q1 is positively associated with the levels of Aurora-A and SAC proteins in colorectal cancer. Our study shows that hnRNP Q1 plays a potentially oncogenic role during tumorigenesis.

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